

(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT &amp; TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER  
110391

**TRANSMITTAL LETTER TO THE  
UNITED STATES  
DESIGNATED/ELECTED OFFICE  
(DO/EO/US) CONCERNING A FILING  
UNDER 35 U.S.C. 371**

U.S. APPLICATION NO.  
(if known, sec 37 C.F.R.1.5)

09/936266

INTERNATIONAL APPLICATION NO.  
PCT/EP00/02011INTERNATIONAL FILING DATE  
MARCH 8, 2000PRIORITY DATE CLAIMED  
MARCH 9, 1999TITLE OF INVENTION  
METHOD FOR IN-VITRO TESTING OF ACTIVE-SUBSTANCES, DEVICE, AND ITS USEAPPLICANT(S) FOR DO/EO/US  
Herna GLOCKNER; Horst-Dieter LEMKE; and Christoph MEYER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern other document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
   
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ Entitlement to small entity status is hereby asserted.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known) <u>37</u> C.F.R. 1.51 <u>26266</u>	INTERNATIONAL APPLICATION NO PCT/EP00/02011	ATTORNEY'S DOCKET NUMBER 110391
---	--	------------------------------------

17. <input checked="" type="checkbox"/> The following fees are submitted:  <b>Basic National fee (37 CFR 1.492(a)(1)-(5)):</b>  Search Report has been prepared by the EPO or JPO .... \$860.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$690.00  No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$710.00  Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1,000.00  International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$ 100.00  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>	<b>CALCULATIONS</b>	<b>PTO USE ONLY</b>																																																						
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$ 860  \$ 0																																																							
<table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:20%;">Claims</th> <th style="width:20%;">Number Filed</th> <th style="width:10%;">Number Extra</th> <th style="width:10%;">Rate</th> <th style="width:10%;"></th> <th style="width:10%;"></th> </tr> </thead> <tbody> <tr> <td>Total Claims</td> <td>46 - 20 =</td> <td>26</td> <td>X \$ 18.00</td> <td>\$ 468</td> <td></td> </tr> <tr> <td>Independent Claims</td> <td>2 - 3 =</td> <td>0</td> <td>X \$ 80.00</td> <td>\$ 0</td> <td></td> </tr> <tr> <td colspan="3">Multiple dependent claim(s) (if applicable)</td> <td>+ \$270.00</td> <td>\$ 0</td> <td></td> </tr> <tr> <td colspan="4"><b>TOTAL OF ABOVE CALCULATIONS =</b></td> <td>\$1328</td> <td></td> </tr> <tr> <td colspan="4">Reduction by 1/2 for filing by small entity, if applicable.</td> <td>- \$</td> <td></td> </tr> <tr> <td colspan="4"><b>SUBTOTAL =</b></td> <td>\$1328</td> <td></td> </tr> <tr> <td colspan="4">Processing fee of \$130.00 for furnishing the English translation later            than <input type="checkbox"/> 20 <input type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR            1.492(f)).</td> <td>\$ 0</td> <td></td> </tr> <tr> <td colspan="4"><b>TOTAL NATIONAL FEE =</b></td> <td>\$1328</td> <td></td> </tr> </tbody> </table>	Claims	Number Filed	Number Extra	Rate			Total Claims	46 - 20 =	26	X \$ 18.00	\$ 468		Independent Claims	2 - 3 =	0	X \$ 80.00	\$ 0		Multiple dependent claim(s) (if applicable)			+ \$270.00	\$ 0		<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$1328		Reduction by 1/2 for filing by small entity, if applicable.				- \$		<b>SUBTOTAL =</b>				\$1328		Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0		<b>TOTAL NATIONAL FEE =</b>				\$1328		Amount to be refunded \$  Charged \$	
Claims	Number Filed	Number Extra	Rate																																																					
Total Claims	46 - 20 =	26	X \$ 18.00	\$ 468																																																				
Independent Claims	2 - 3 =	0	X \$ 80.00	\$ 0																																																				
Multiple dependent claim(s) (if applicable)			+ \$270.00	\$ 0																																																				
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$1328																																																				
Reduction by 1/2 for filing by small entity, if applicable.				- \$																																																				
<b>SUBTOTAL =</b>				\$1328																																																				
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0																																																				
<b>TOTAL NATIONAL FEE =</b>				\$1328																																																				

a. <input checked="" type="checkbox"/> Check No. <u>122550</u> in the amount of \$ <u>1,328</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. <u>    </u> in the amount of \$ <u>    </u> to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. <u>15-0451</u> . A duplicate copy of this sheet is enclosed.	NAME: <u>William B. Berridge</u> REGISTRATION NUMBER: <u>30,024</u>  NAME: <u>Christopher W. Brown</u> REGISTRATION NUMBER: <u>38,025</u>  NAME: <u>Joel S. Armstrong</u> REGISTRATION NUMBER: <u>36,430</u>
---	---

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:  
 OLUFF & BERRIDGE, PLC  
 P.O. Box 19928  
 Alexandria, Virginia 22320

Date: September 10, 2001

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Herma GLOCKNER et al.

Application No.: New U.S. National Stage Application  
of PCT/EP 00/02011

Filed: September 10, 2001

Docket No.: 110391

For: METHOD FOR IN-VITRO TESTING OF ACTIVE SUBSTANCES, DEVICE, AND  
ITS USE

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office  
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE ABSTRACT:

Please replace the Abstract filed with the attached Abstract hereto.

IN THE SPECIFICATION:

Page 1, delete "AGW2503" in the upper left corner.

Please replace paragraph [0001] as follows:

--BACKGROUND OF THE INVENTION

1. Field of the Invention--

Please replace paragraph [0002] as follows:

The invention relates to a method for testing active substances on cells in-vitro, a device, and a process for testing employing the device.

Page 1, after paragraph [0002], please insert new paragraph [0002.1] as follows:

--2. Discussion of Related Art--

Page 4, after paragraph [0010], please insert new paragraph [0010.1] as follows:

--SUMMARY OF THE INVENTION--

Page 5, after paragraph [0013], please insert new paragraphs [0013.1] - [0013.8] as follows:

[0013.1] --BRIEF DESCRIPTION OF DRAWING

[0013.2] Figure 1 is a flowchart of a device of the invention.

[0013.3] Figure 2a is a cross section through a modular active substance testing system with devices of the invention.

[0013.4] Figure 2b is a cross section through a modular active substance testing system with three stacked planes with devices of the invention. and

[0013.5] Figure 3 is a top view of a modular active substance testing system comprising six devices of the invention.

[0013.6] Figure 4a is an active substance concentration-time curve shown as Profiles 1 to 3.

[0013.7] Figure 4b shows the vital cells in four different cell culture containers as presented (inoculum) and as harvested cells (cell harvest).

[0013.8] DESCRIPTION OF PREFERRED EMBODIMENTS--

Please delete paragraphs [0058] - [0063].

Top page 22, delete " Method for In-vitro Testing of Active Substances, Device and Its Use"

Page 22, replace "Claims:" with "What is claimed is:"

IN THE CLAIMS:

Please amend the claims, as shown in the Annex to the International Preliminary Examination Report (IPER), as follows:

Please replace claims 1-45 as follows:

1. (Amended) A method for in-vitro testing of active substances in cells comprising at least the following steps:
  - a) providing a cell culture container with an interior chamber and an inside wall and with a first and second membrane system located in the interior chamber, whereby a cell culture space is formed between the first and second membrane systems and the inside wall of the interior chamber;
  - b) providing cells as a cell culture and a cell culture medium in the cell culture space;
  - c) adding a fluid nutrient medium to the cell culture space and removing metabolic products from the cell culture space by means of the first membrane system;
  - d) adding at least one gaseous medium to the cell culture space by means of the second membrane system;
  - e) metering at least one active substance into the cell culture space, with the metering taking place according to an adjusted active substance concentration-time curve; and
  - f) monitoring cell vitality.
2. (Amended) The method according to claim 1, wherein the active substances comprise cytostatics, antibiotics, cytokines, growth factors, or antiviral agents.
3. (Amended) The method according to claim 1, wherein the cell culture comprises primary cells.
4. (Amended) The method according to claim 1, wherein the cell culture comprises tumor cell lines.

5. (Amended) The method according to claim 1, wherein the cell culture space has a minimum volume of at least 0.1 ml and a maximum volume of 5 ml.

6. (Amended) The method according to claim 5, wherein the cell culture space has a minimum volume of 0.3 ml and a maximum volume of 3.0 ml.

7. (Amended) The method according to claim 1, wherein the first membrane system comprises at least one semipermeable membrane or at least one hydrophilic microporous membrane, and the second membrane system comprises at least one gas transfer membrane.

8. (Amended) The method according to claim 1, wherein the first and the second membrane systems comprise hollow fibers stacked in multiple layers.

9. (Amended) The method according to claim 1, wherein the cell culture container comprises a removable lid and the cell culture is provided by adjusting a desired cell density in the cell culture medium, opening the removable lid of the cell culture container, pipetting a desired volume of cell suspension into the cell culture container, and closing the removable lid of the cell culture container so as to close the cell culture container.

10. (Amended) The method according to claim 1, wherein the cell culture medium comprises RPMI 1640.

11. (Amended) The method according to claim 1, wherein the cell culture space comprises at least  $1 \cdot 10^5$  cells per ml.

12. (Amended) The method according to claim 1, wherein each cell is at an average distance of 0  $\mu\text{m}$  to 600  $\mu\text{m}$  from the closest membrane in the first and second membrane systems.

13. (Amended) The method according to claim 1, wherein a fluid nutrient medium comprises RPMI 1640.

14. (Amended) The method according to claim 1, wherein the gaseous medium comprises a  $pO_2$  of 0 to 160 mmHg and a  $pCO_2$  of 0 to 115 mmHg.

15. (Amended) The method according to claim 14, wherein the cell culture medium comprises a bicarbonate buffer and the  $pCO_2$  in the gaseous medium is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

16. (Amended) The method according to claim 1, wherein the second membrane system removes gaseous metabolic products from the cell culture space.

17. (Amended) The method according to claim 1, wherein the metering of the at least one active substance comprises adding at least one active substance on a time-staggered basis.

18. (Amended) The method according to claim 1, wherein the metering of the at least one the active substance comprises adding a dose of the at least one active substance to the cell culture space directly or through the first membrane system.

19. (Amended) The method according to claim 1, wherein the active substance concentration-time curve is determined based on permeabilities of the first membrane system, duration of the active substance administration, and active substance concentration.

20. (Amended) The method according to claim 1, wherein the cell culture container is kept at 37°C.

21. (Amended) The method according to claim 1, wherein monitoring of cell vitality comprises measuring the presence of fluorescent dye converted from a cell vitality dye.

22. (Amended) The method according to claim 21, wherein the cell vitality dye comprises Alamar Blue.

23. (Amended) The method according to claim 1, wherein the monitoring of cell vitality comprises at least one sensor.

24. (Amended) The method according to claim 23, wherein the sensor comprises a fluorescence sensor.

25. (Amended) A device for in-vitro testing of active substances in cells, comprising a cell culture container suitable for collecting a cell culture in a cell culture medium with an interior chamber, wherein a first supply device for introducing at least one nutrient medium and a second supply device for adding at least one gaseous medium are located in the interior chamber, wherein each supply device has a supply side and a removal side, and a cell culture space being formed between said supply devices and an inside wall of the interior chamber, and with the first supply device in a fluid connection with the supply side connected to a nutrient medium dispensing unit with at least one nutrient medium container, and the second supply device connected in a fluid connection with the supply side connected to a gas metering unit with at least one gas supply container, wherein the cell culture space has a volume of at most 5 ml and at least 0.1 ml, and further wherein the device comprises an active substance supply container, an active substance dispensing unit, a line system connecting the active substance supply container with the interior chamber for supplying at least one active substance to the cell culture space, and wherein the active substance dispensing unit dispenses the active substance into the cell culture space according to an adjusted active substance concentration-time curve.

26. (Amended) The device according to claim 25, wherein the first supply device includes a fluid connection on the removal side with a waste container.

27. (Amended) The device according to claim 25, wherein the first supply device includes a fluid connection on the removal side by a recirculation line comprising at least one nutrient medium container.

28. (Amended) The device according to claim 25, wherein the first supply device comprises at least one membrane suitable for supplying nutrient media.

29. (Amended) The device according to claim 25, wherein the second supply device comprises at least one membrane suitable for gas exchange.

30. (Amended) The device according to claim 25, wherein the cell culture container comprises a bottom and a lid binding the interior chamber, being opposite one another, and each comprising a transparent material.

31. (Amended) The device according to claim 30, wherein the bottom of the cell culture container includes a heating system.

32. (Amended) The device according to claim 25, wherein the first supply device comprises at least one membrane that is a semipermeable membrane or a hydrophilic microporous membrane.

33. (Amended) The device according to claim 25, wherein the second supply device comprises at least one membrane that is an oxygenation membrane.

34. (Amended) The device according to claim 25, wherein the first and second supply devices comprise membranes that are hollow fibers.

35. (Amended) The device according to claim 34, wherein the hollow fibers are stacked in several layers in the interior chamber.

36. (Amended) The device according to claim 35, wherein the maximum distance between the hollow fibers forming each supply device is between 50  $\mu\text{m}$  and 600  $\mu\text{m}$ .

37. (Amended) The device according to claim 25, wherein the cell culture space comprises a volume of 0.3 ml to 3.0 ml.

38. (Amended) The device according to claim 25, wherein the supply device for adding the active substance comprises at least one active substance supply container, at least one active substance metering device, and a system of lines which connects the at least one active substance supply container through the at least one active substance metering

device directly or through the first supply device with the cell culture space of the cell culture container.

39. (Amended) The device according to claim 25, wherein the device further includes a monitor for cell vitality.

40. (Amended) The device according to claim 39, wherein the monitoring of cell vitality comprises at least one sensor.

41. (Amended) The device according to claim 40, wherein the sensor comprises a fluorescence sensor.

42. (Amended) A modular active substance testing system comprising at least two devices according to claim 25.

43. (Amended) The modular active substance testing system according to claim 42, comprising 6, 24, or 96 devices.

44. (Amended) A process for in-vitro testing of the effects of active substances on cells comprising the device according to claim 25.

45. (Amended) The process according to claim 44, wherein the process comprises determining the influence of pharmacokinetics on cell vitality.

Please add new claim 46 as follows:

--46. A process for in-vitro testing of the effects of active substances on cells comprising the modular active testing substance according to claim 42.--

#### REMARKS

Claims 1-46 are pending. By this Preliminary Amendment, the specification and claims are conformed to U.S. practice, including adding section headings and addressing antecedent basis issues. Accordingly, no new matter is added by this Preliminary Amendment.

A prompt and favorable examination on the merits is earnestly solicited. The Examiner is invited to contact the undersigned representative to discuss any matter with respect to this application.

Respectfully submitted,



William P. Berridge  
Registration No. 30,024

Christopher W. Brown  
Registration No. 38,025

WPB:CWB:DML/rxg

Date: September 10, 2001

Attachments:

Appendix  
Abstract

**OLIFF & BERRIDGE, PLC**  
**P.O. Box 19928**  
**Alexandria, Virginia 22320**  
**Telephone: (703) 836-6400**

<p>DEPOSIT ACCOUNT USE AUTHORIZATION Please grant any extension necessary for entry; Charge any fee due to our Deposit Account No. 15-0461</p>
--

## APPENDIX

## Changes to Abstract:

The following is a marked-up version of the amended Abstract.

**Method for In-vitro Testing of Active Substances, Device and Its Use**

## Summary:

ABSTRACT

A method for in-vitro testing of active substances on cells ~~comprises at least~~ includes the first steps ~~for~~ of providing a cell culture container with an interior chamber, and an inside wall, and ~~with a~~ first and second membrane system located in the interior chamber, ~~with a~~ A cell culture chamber is formed between the membrane systems and the inside wall of the interior ~~space~~ chamber. The method includes providing a cell culture and cell culture medium in a cell culture chamber, supplying a fluid nutrient medium to the cell culture chamber, and carrying away metabolic products by means of the first membrane system, ~~adding at~~ At least one gaseous medium is added to the cell culture chamber by means of the second membrane system, ~~adding and~~ at least one active substance is added to the cell culture chamber, ~~with the supplying taking~~ The supplying takes place according to an adjusted active substance concentration-time curve and monitoring cell vitality. In addition, a device and the use of said device for testing the effect of idarubicin on the leukemic cell line CCRF CEM is described.

## Changes to Specification:

The following is a marked-up version of the amended paragraphs:

**[0001] Description:**BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The invention relates to a method for testing active substances on cells in-vitro, a device, and its use a process for testing employing the device.

Changes to Claims:

The following is a marked-up version of the amended claims:

1. (Amended) ~~Use of a~~ A method for in-vitro testing of active substances in cells comprising at least the following steps:
    - a) ~~Providing~~ providing a cell culture container with an interior ~~space~~ chamber and an inside wall and with a first and second membrane system located in the interior ~~space~~ chamber, whereby a cell culture space is formed between the first and second membrane systems and the inside wall of the interior chamber;
    - b) ~~Providing~~ providing cells as a cell culture and a cell culture medium in the cell culture ~~chamber~~ space;
    - c) ~~Adding~~ adding a fluid nutrient medium to the cell culture ~~chamber~~ space and removing metabolic products from the cell culture ~~chamber~~ space by means of the first membrane system;
    - d) ~~Adding~~ adding at least one gaseous medium to the cell culture ~~chamber~~ space by means of ~~a~~ the second membrane system;
    - e) ~~Metering~~ metering at least one active substance into the cell culture ~~chamber~~ space, with the metering taking place according to an adjusted active substance concentration-time curve; and
    - f) ~~Monitoring~~ monitoring cell vitality.
- ~~for in-vitro testing of active substances in cells.~~

2. (Amended) Use The method according to Claim claim 1, echaracterized in that wherein the active substances comprise cytostatics, antibiotics, cytokines, growth factors, or antiviral agents ~~are used as active substances.~~

3. (Amended) Use The method according to ~~one or more of Claims claim 1 or 2~~, echaracterized in that wherein the cell culture comprises primary cells ~~are added as the cell culture.~~

4. (Amended) Use The method according to ~~one or more of Claims claim 1 or 2~~, echaracterized in that wherein the cell culture comprises tumor cell lines ~~are used as the cell culture.~~

5. (Amended) Use The method according to ~~one or more of Claims claim 1 to 4~~, echaracterized in that wherein the cell culture chamber space has a minimum volume of at least 0.1 ml ~~minimum~~ and a maximum volume of 5 ml ~~maximum~~.

6. (Amended) Use The method according to Claim claim 5, echaracterized in that wherein the cell culture chamber space has a minimum volume of 0.3 ml and a maximum volume of 3.0 ml.

7. (Amended) Use The method according to ~~one or more of Claims claim 1 to 6~~, echaracterized in that wherein the first membrane system comprises at least one semipermeable membrane or at least one hydrophilic microporous membrane ~~is used as the first membrane system~~, and the second membrane system comprises at least one gas transfer membrane ~~is used as the second membrane system.~~

8. (Amended) Use The method according to ~~one or more of Claims claim 1 to 7~~, echaracterized in that wherein the first and the second membrane systems consist of comprise hollow fibers stacked in multiple layers.

9. (Amended) Use The method according to ~~one or more of Claims claim 1 to 8, characterized in that a~~ wherein the cell culture container is used which has comprises a removable lid and allows the cell culture to be prepared is provided by adjusting the a desired cell density in the cell culture medium, opening the removable lid of the cell culture container, pipetting the a desired volume of cell suspension into the cell culture container, and closing the removable lid of the cell culture container using the lid so as to close the cell culture container.

10. (Amended) Use The method according to ~~one or more of Claims claim 1 to 9, characterized in the fact that~~ wherein the cell culture medium comprises RPMI 1640 is used as the cell culture medium.

11. (Amended) Use The method according to ~~one or more of Claims claim 1 to 10, characterized in that~~ wherein the cell culture space comprises at least  $1 \cdot 10^5$  cells per ml of cell culture space are used.

12. (Amended) Use The method according to ~~one or more of Claims claim 1 to 11, characterized in that~~ wherein each cell is at an average distance of 0  $\mu$ m to 600  $\mu$ m from the closest membrane in the first and second membrane systems.

13. (Amended) Use The method according to ~~one or more of Claims claim 1 to 12, characterized in that~~ wherein a fluid nutrient medium comprises RPMI 1640 is used.

14. (Amended) Use The method according to ~~one or more of Claims claim 1 to 13, characterized in that~~ wherein the gaseous medium has comprises a  $pO_2$  of 0 to 160 mmHg and a  $pCO_2$  of 0 to 115 mmHg.

15. (Amended) Use The method according to ~~one or more of Claims claim 1 to 14, characterized in that~~ wherein the cell culture medium contains comprises a bicarbonate

buffer and the  $p\text{CO}_2$  in the gaseous medium added is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

16. (Amended) Use The method according to ~~one or more of Claims claim 1 to 15, characterized in that wherein~~ gaseous metabolic products are removed from the cell culture space by means of the second membrane system removes gaseous metabolic products from the cell culture space.

17. (Amended) Use The method according to ~~one or more of Claims claim 1 to 16, characterized in that wherein the metering of the at least one individual active substances and/or combinations of several active substances are added~~ substance comprises adding the at least one active substance on a time-staggered basis.

18. (Amended) Use The method according to ~~one or several of Claims claim 1 to 17, characterized in that wherein the metering of the at least one active substance dosage is added~~ comprises adding a dose of the at least one active substance to the cell culture chamber space directly or by means of through the first membrane system.

19. (Amended) Use The method according to ~~one or more of Claims claim 1 to 18, characterized in that specification of wherein~~ the active substance concentration-time curve ~~takes place with the~~ is determined based on permeabilities of the first membrane system, ~~by the~~ duration of the active substance administration, and ~~by the~~ active substance concentration.

20. (Amended) Use The method according to ~~one or more of Claims claim 1 to 19, characterized in that wherein~~ the cell culture container is kept at 37°C.

21. (Amended) Use The method according to ~~one or more of Claims claim 1 to 18, characterized in that wherein monitoring of~~ the cell vitality ~~is monitored by means of~~ comprises measuring the presence of fluorescent dye converted from a cell vitality dye.

22. (Amended) Use The method according to ~~Claim claim~~ claim 21, characterized in that wherein the cell vitality dye comprises Alamar Blue serves as a cell vitality dye

23. (Amended) Use The method according to ~~one or more of Claims claim~~ claim 1 to 22, characterized in the fact wherein the monitoring of cell vitality is monitored using comprises at least one sensor.

24. (Amended) Use The method according to ~~Claim claim~~ claim 23, characterized in that wherein the sensor comprises a fluorescence sensor is used.

25. (Amended) Device A device for in-vitro testing of active substances in cells, comprising a cell culture container (1) suitable for collecting a cell culture in a cell culture medium with an internal interior chamber (2), with wherein a first means for supplying supply device for introducing at least one nutrient medium and a second supply device for adding at least one gaseous medium are located in the interior space chamber, with the means each having wherein each supply device has a supply side and a removal side, and with a cell culture space being formed between said means supply devices and the an inside wall of the interior chamber, and with the first means supply device in a fluid connection with the supply side connected by to nutrient medium dispensing unit (3) with including at least one nutrient medium container (4), and the second means supply device connected in a fluid connection with the supply side connected by to a gas metering unit (5) with including at least one gas supply container (6), characterized in that wherein the cell culture chamber space has a volume of at most 5 ml and at least 0.1 ml, and that further wherein the device also contains means (7), (8), (9a), (9b), and (9e) comprises an active substance supply container, an active substance dispensing unit, and a line system connecting the active substance supply container with the interior chamber for supplying at least one active substance to the cell culture chamber space, and means for creating an active substance wherein the active substance

dispensing unit dispenses the active substance into the cell culture space according to an adjusted active substance concentration-time curve in the cell culture chamber.

26. (Amended) ~~Device~~ The device according to Claim claim 25, characterized in that wherein the first means is in supply device includes a fluid connection on the removal side with a waste container (40).

27. (Amended) ~~Device~~ The device according to Claim claim 25, characterized in that wherein the first means is in supply device includes a fluid connection on the removal side by a recirculation line (41) ~~with the~~ comprising at least one nutrient medium container (4).

28. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 27, characterized in that wherein the first means consists of supply device comprises at least one ~~fluid medium suitable for administration~~ membrane suitable for supplying nutrient media.

29. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 28 characterized in that wherein the second means supply device consists of comprises at least one membrane suitable for gas exchange.

30. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 29, characterized in that wherein cell culture container (1) ~~has~~ comprises a bottom and a lid ~~which bound~~ binding the interior chamber, being are opposite one another, and consist of each comprising a transparent material.

31. (Amended) ~~Device~~ The device according to Claim claim 30, characterized in that wherein the bottom of the cell culture container includes a heating system ~~is integrated into the bottom of cell culture container (1).~~

32. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25, ~~characterized in that the~~ wherein the first supply device comprises at least one membrane ~~of the first means~~ that is a semipermeable membrane or a hydrophilic microporous membrane.

33. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 32, ~~characterized in that~~ wherein the second supply device comprises ~~the~~ at least one membrane ~~of the second means~~ that is an oxygenation membrane.

34. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 33, ~~characterized in that~~ wherein the first and second supply devices comprise ~~the~~ membranes ~~of the first and second means~~ that are hollow fibers.

35. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 34, ~~characterized in that~~ wherein the hollow fibers are stacked in several layers in the interior chamber.

36. (Amended) ~~Device~~ The device according to ~~Claim claim~~ 35, ~~characterized in that~~ wherein the maximum distance between the hollow fibers forming each ~~means~~ supply device is between 50 ~~in~~ µm and 600 ~~in~~ µm.

37. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 36, ~~characterized in that~~ wherein the cell culture chamber has ~~space~~ comprises a volume of 0.3 ml to 3.0 ml.

38. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 37, ~~characterized in that~~ wherein the ~~means~~ supply device for adding the active substance ~~consist of~~ comprises at least one active substance supply container (7), at least one active substance metering device (8), and a system of lines (9) which connects the at least one active substance supply container (7) through ~~an~~ the at least one active substance metering

~~unit (8) device~~ directly ~~(9a)~~ or through ~~first means (9b)~~ the first supply device with the ~~cell culture chamber~~ cell culture space of the cell culture container (1).

39. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim 25 to 38, characterized in that wherein~~ the device has ~~a means for monitoring~~ further includes monitoring for cell vitality.

40. (Amended) ~~Device~~ The device according to Claim claim 39, ~~characterized in that wherein~~ the ~~means for monitoring~~ monitoring of cell vitality ~~consists of comprises~~ at least one sensor.

41. (Amended) ~~Device~~ The device according to Claim claim 40, ~~characterized in that wherein~~ the sensor is comprises a fluorescence sensor.

42. (Amended) ~~Modular~~ A modular active substance testing system comprising at least two devices according to Claims claim 25 to 41.

43. (Amended) ~~Modular~~ The modular active substance testing system according to Claim claim 42, ~~consisting of comprising~~ 6, 24, or 96 devices according to Claims 25 to 41.

44. (Amended) ~~Use of the device according to one or more of Claims 25 to 41 or of the modular active substance testing system according to one of Claims 42 or 43 for in vitro testing of the effects of active substances on cells~~ A process for in-vitro testing of the effects of active substances on cells comprising the device according to claim 25.

45. (Amended) ~~Use of the device or of the modular system according to Claim 44, characterized in that~~ The process according to claim 44, wherein the process comprises determining the influence of pharmacokinetics on cell vitality is determined.

ABSTRACT

A method for in-vitro testing of active substances on cells includes the first step of providing a cell culture container with an interior chamber, an inside wall, and a first and second membrane system located in the interior chamber. A cell culture chamber is formed between the membrane systems and the inside wall of the interior chamber. The method includes providing a cell culture and cell culture medium in a cell culture chamber, supplying a fluid nutrient medium to the cell culture chamber, and carrying away metabolic products by means of the first membrane system. At least one gaseous medium is added to the cell culture chamber by means of the second membrane system, and at least one active substance is added to the cell culture chamber. The supplying takes place according to an adjusted active substance concentration-time curve and monitoring cell vitality. In addition, a device and the use of said device for testing the effect of idarubicin on the leukemic cell line CCRF CEM is described.

METHOD FOR IN-VITRO TESTING OF ACTIVE SUBSTANCES,  
DEVICE, AND ITS USE

**[0001]** Description:

**[0002]** The invention relates to a method for testing active substances on cells in-vitro, a device, and its use.

**[0003]** Testing active substances such as cytostatics, for example, that can be used in cancer chemotherapy, is necessary for many reasons. A distinction must be made as to whether a completely new active substance is involved, or whether an active substance whose effectiveness is already basically known is involved. In the case of an unknown active substance, the basic effectiveness, for example, within the scope of an active substance screening, and the dose and the optimum application form, for example, oral or intravenous, must be determined first. In the case of already-known active substances, for example, possible therapeutic strategies are tested and compared with one another. Another important issue is the patient-specific testing or patient-specific effects of a substance on an individual patient. For each of these questions different model systems are available.

**[0004]** For active substance screening of unknown substances, work is performed without exception, not on patients due to ethical reasons, but on animal models in-vivo or in-vitro with cell lines. Animal models for screening unknown cytostatics are based on the fact that for each treatment of the receiving animal human tumor cell samples are encapsulated in hollow fiber devices and implanted in the receiving animal, as described in WO 94/25074 and U.S. Patent No. 5,676,924. This procedure has its limits, however, since animal models are naturally difficult to automate and are associated with great effort and high costs. Increasingly, the idea of animal protection counteracts testing in animal models.

**[0005]** The transferability of the test results in experimental animals, typically rodents, to man is also a question. An important role is played by metabolic differences between humans and animal species, leading to different pharmacokinetics in animal models. Within the scope of this invention, pharmacokinetics is understood to be the dynamic process by which an active substance is absorbed with different kinetics in the organism, thus absorbed, distributed, metabolized, i.e., reacted and excreted again. These processes cannot be quantified independently of one another in-vivo since they overlap in time and take place in mutual dependence. In the Compendium of Internal Oncology, published by H.J. Schmoll, K. Höffgen, K. Possinger, Springer Verlag (1996), mathematical models were therefore used, which describe the distribution of a medication in theoretical body spaces or compartments. In particular, in addition to active substance properties and metabolism, the form of application acts on the pharmacokinetics of the substance. The pharmacokinetics of an active substance are usually determined in-vivo by measuring the serum concentration as a function of time.

**[0006]** The use of in-vitro models for testing substances is also widespread. Cultivation of human primary cells in monolayer cultures for patient-specific testing is preferred to achieve a high sample throughput. Such a procedure was described, for example, by G.J.L. Kaspers et al. in Blood (1997), Volume 90, No. 7, pages 2723-29 and by R. Pieters in Blood (1990), Volume 76, No. 11, pages 76, 2327-36. The disadvantage of known in-vitro methods is that the microtiter plates used in them do not allow three-dimensional growth of the cells. Solid tumors, for example, develop different subpopulations probably because of gradients in the pH value and the nutrient supply. These gradients cause regional variations in cell vitality, metabolism, and sensitivity to treatment with cytostatics. It has been suggested that malignant cells

in a three-dimensional tissue-like structure have a different response to cytostatics than monolayer cultures, as follows from J.J. Casciari et al., J. Natl. Cancer Inst. (1994), Volume 86, pages 1846-52 and K.M. Nicholson et al., Ann. Oncology (1996), Volume 7, Supplement 1, Abstract.

[0007] Another disadvantage of known in-vitro methods is that the pharmacokinetics that take place in the human organism cannot be replicated in-vitro as yet, thus they cannot be modeled by corresponding in-vitro pharmacokinetics. However, the scope of the present invention covers in-vitro pharmacokinetics. This means that the active substance concentration in the vicinity of the target cells changes as a function of the time in-vitro in the same way as in the in-vivo environment of the target cells, as in leukemias in the serum, regardless of what means achieve this goal. The primary criterion is, therefore, the shape of the curve and the absolute value of the active substance concentration, while the time axis can be tightened up or stretched out relative to the in-vivo situation.

[0008] A third disadvantage of all of the in-vitro methods known today for active substance testing is that no combination therapy can be simulated. This is, therefore, of critical importance because today only in exceptional cases the treatment is performed with only one cytostatic, i.e., with a monotherapy. So, most therapies are combination therapies, in which a time sequence of different cytostatics takes place, as in the Compendium of Internal Oncology, published by H.J. Schmoll, K. Höffgen, K. Possinger, Springer Verlag (1996). In the standard in-vitro method, an active substance that has been introduced into the assay cannot be removed again as it is possible in the organism before the next active substance is given.

[0009] All three of these disadvantages lead to the fact that currently the response of real tumors to chemotherapy in-vitro cannot be simulated very well. This

leads to serious doubts among experts about the prognostic value of the in-vitro methods known thus far for testing active substances in cells.

[0010] So, there is a need for an in-vitro method for testing active substances on cells and for a device that can be used for this method, in order that the above-mentioned disadvantages can be at least considerably reduced.

[0011] The present invention, therefore, has the goal of providing an in-vitro method for testing active substances on cells, and a device that can be used for this purpose so that the disadvantages mentioned above can be at least considerably reduced and an approximation to in-vivo pharmacokinetics can be achieved by suitable in-vitro pharmacokinetics, and combination therapy with different active substances can be simulated.

[0012] This goal is achieved by a method for in-vitro testing of active substances on cells comprising at least the following steps:

- a) a cell culture container with an interior chamber and an inside wall and with a first and second membrane system located in the interior chamber are made available, with a cell culture space being formed between the membrane systems and the inside wall of the interior chamber;
- b) providing a cell culture and a cell culture medium in the cell culture space;
- c) adding a fluid nutrient medium to the cell culture space and removing metabolic products by the first membrane system;
- d) adding at least one gaseous medium to the cell culture space by the second membrane system;

e) metering at least one active substance to the cell culture space, wherein the addition taking place according to an adjusted active substance concentration-time curve; and

f) monitoring cell vitality.

**[0013]** The active substances to be tested include those substances within the scope of the present invention whose effect on the cells to be investigated is unknown or is insufficiently known before the test. These can be gaseous active substances such as respiratory poisons or other optionally toxic gases. Among these substances defined above, cytostatics, antibiotics, cytokines, growth factors, or antiviral agents are preferably used. In addition, basically all of the substances as defined above can be tested for their effect on cells when these substances can be added in dissolved form to the cell culture space.

**[0014]** Cell cultures can be tested using the method of the invention that consist preferably of primary cells or of cell lines, more preferably employing tumor cell lines. The volume of the cell culture space is preferably 0.3 ml minimum and 2.0 ml maximum, and more preferably between 0.5 ml and 1.5 ml. As a result, only a small amount of cell material is used so that, for example, only small amounts of cells need to be removed from a cancer patient for the method of the invention.

**[0015]** The first membrane system located inside the cell culture container consists of at least one semipermeable membrane, suitable for the addition of a liquid nutrient medium, i.e., a continuous material transport is permitted through the membrane wall by diffusive or convective transport mechanisms. Depending on the requirement, or depending on whether diffusive or convective material transport of the nutrient medium is necessary, nanofiltration, ultrafiltration, or microfiltration membranes are used. For example, dialysis membranes such as CUPROPHAN® or

hydrophilic microporous membranes such as microporous polyethersulfone membranes are used. The second membrane system in the interior of the cell culture container consists of at least one gas transfer membrane, preferably an oxygenation membrane like that commercially available as OXYPHAN®. The membranes of the first and second membrane system are preferably hollow fiber membranes.

[0016] In a preferred embodiment of the method of the invention, a cell culture container comprises the first and second membrane systems made of hollow fibers, which are stacked in multiple layers in the interior chamber.

[0017] The presentation of the cell culture takes place in a preferred embodiment of the method of the invention, when using a cell culture container with a lid by adjusting the cell density in the cell culture medium, opening the lid of the cell culture container, pipetting the desired volume of the cell suspension into the interior of the cell culture container, and closing the cell culture container with the lid.

[0018] In another preferred embodiment of the method of the invention, the cell culture can be introduced through an opening in a side wall of the interior chamber which is provided, for example, with a connection for a syringe, or the like, on the outside of the cell culture container.

[0019] In a third preferred embodiment of the method of the invention, the cell culture can be added through at least one septum through which access to the interior of the cell culture container is possible.

[0020] The cells can be in the form of suspension cells or adhesion-requiring cells, with suspension cells, as a rule, being the cells that come from the blood, and adhesion-requiring cells, as a rule, being those cells that come from the body tissue. The latter can either be placed directly as undivided pieces of tissue in the interior of the cell culture container, or the pieces of tissue are broken up first, and then used as

suspension cells. For adhesion-requiring cells, the interior of the cell culture container is advantageously equipped with a surface to which the cells preferentially adhere. Preferably, this surface consists of protein-coated polycarbonate, or of textile material made of polyester additionally placed in the interior of the cell culture container.

[0021] When the method of the invention is performed, the cell culture container preferably contains at least  $1 \cdot 10^5$  cells per ml of cell culture space, more preferably at least  $1 \cdot 10^6$  cells per ml of cell culture space. In an especially suitable version, the cell density in the cell culture space is more than  $5 \cdot 10^7$  cells per ml of cell culture space. In a preferred embodiment of the method of the invention, suspension cells with a cell density of at least  $1 \cdot 10^5$  cells per ml of cell culture space are used. An approximation of the cell density value in the blood is possible in this way. In addition, when using adhesion-requiring cells, the method of the invention preferably comprises a cell density of at least  $1 \cdot 10^5$  cells per ml of cell culture space in order to approximate the cell density of body tissue after the corresponding cell growth.

[0022] Advantageously, each cell has an average distance of 0  $\mu\text{m}$  to 600  $\mu\text{m}$  from the closest membrane of the first and second membrane systems. Thereby, the cells are uniformly supplied with nutrient medium and gas. At the same time, metabolic products are carried away uniformly so a state that resembles in-vivo conditions is simulated in a cell culture.

[0023] Media that are usually employed for supplying cells with nutrient substances or for growing cells can be used as the fluid nutrient medium or as the cell culture medium. Preferably, the cell culture medium comprises RPMI 1640. In a more preferred embodiment of the method of the invention, RPMI 1640 and fetal calf serum containing nutrient medium are used.

**[0024]** A gas mixture is preferred as the gaseous medium having an oxygen partial pressure  $pO_2$  of 0 to 160 mmHg, and a carbon dioxide partial pressure  $pCO_2$  from 0 to 115 mmHg. In a preferred embodiment of the method of the invention, a cell culture medium contains a bicarbonate buffer and the  $pCO_2$  in the introduced gaseous medium is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

**[0025]** The desired composition of the gaseous medium can be adjusted by the method of the invention in a space where this composition prevails. In this case, the gaseous medium is added through a sterile filter to the second membrane system.

**[0026]** The second membrane system can also advantageously be used to remove gaseous metabolic products if it is operated in cross-flow mode, and connected with a gas supply line and a gas removal line.

**[0027]** In one advantageous embodiment of the method, individual active substances and/or combinations of several active substances are added staggered in time. Thus, for example, an individual active substance can be given as a function of time distributed over several sequential doses, or different individual active substances can be added to the cell culture medium in a time-staggered manner. In the same way, a sequence of active substance combinations can be given time-staggered with the active substance combinations remaining the same or changing in their composition. Finally, individual active substances and active substance combinations of the type described above can be given time-staggered in the method of the present invention.

**[0028]** Active substance administration can be direct or through the first membrane system or, when a gaseous active substance is used, by the second membrane system to the cell culture space. When the active substances are added

through the first membrane system, the active substance is added to the nutrient medium flow and enters the cell culture space with the nutrient medium through the membranes of the first membrane system. When a gaseous active substance is added by the second membrane system, the latter is fed into the gaseous medium and together with the gaseous medium enters the cell culture space through the membranes of the second membrane system. This, of course, means that the membranes of the two membrane systems must be permeable to the respective active substance. When the active substance is administered by means of the first or second membrane system, the active substance concentration-time curve, for example, is adjusted by the permeability of the first or second membrane system, by the duration of the active substance administration, and by the active substance concentration. So, the methods described above allow the simulation of very different pharmacokinetics of individual active substances and/or active substance combinations. For example, the active substance administration, analogous to continuous infusion, can be ramped or analogous to an intravenous administration with peaks.

[0029] During the active substance test, the cell culture container is kept at a temperature suitable for growing the cells, preferably 37°C.

[0030] Monitoring of cell vitality is understood within the scope of the present invention to be the monitoring of the metabolic cell activity, proliferation, apoptosis, or cell death in general.

[0031] To monitor cell vitality, in a preferred embodiment of the method of the invention, a cell vitality dye is used. More preferably Alamar Blue® is used. The cell vitality dye can be administered through the nutrient medium and the first membrane system to the cell culture space. Alamar Blue® penetrates the membranes of the cells, enters the interior of the cell, and is converted by metabolic exchange

there to a fluorescent dye. Therefore, the quantity of fluorescent dye formed can be used as a measure of cell vitality and can be detected by a fluorescence sensor online or after removing a sample in the nutrient medium. It is especially advantageous that removal of a sample from the cell culture space is not necessary, but can take place in the fluid flow leaving the cell culture container. A further advantage is the fact that the cell vitality dye is not only added by the first cell membrane system, but also can be removed completely so that all problems with the cell culture caused by the dye can be corrected and reversed.

**[0032]** In a method of the invention, at least one sensor can be used to monitor cell vitality, supplying information about the state of the cells. Preferably, these are miniaturized sensors to determine proliferation, vitality, apoptosis, or cell death in general. More preferably the sensor is a sensor for fluorescence.

**[0033]** In the method according to the invention, suitable sensors can be used to monitor the process. Preferably, sensors for monitoring temperature, pH, partial pressure of oxygen  $pO_2$ , or carbon dioxide  $pCO_2$ , glucose, or lactate are used. In a more preferred embodiment, the sensors are integrated into the interior of the cell culture container as microsensors, with no disturbing influence of the sensors on the cell culture. In addition, one or more of these sensors can be used in the cell culture space or in the nutrient medium and the sensor signals can be followed online. Thus, for example, pH,  $pO_2$ , glucose, and lactate can be measured simultaneously. Instead of, or in addition to the online sensors described above, further preferred analytical methods are used in the method according to the invention for endpoint determination such as MTT assay (mitochondrial reduction of tetrazolium dye) or flow cytometry.

**[0034]** The goal is also achieved by a device which is used to collect the cell culture in a cell culture medium using a suitable cell culture container with an interior

chamber, wherein first means for adding at least one nutrient medium and second means for adding at least one gaseous medium are located in the interior chamber. The means have a supply side and a removal side and a cell culture space is formed between these means and the inside wall of the interior chamber, the first means with its supply side is linked in a fluid connection by a nutrient medium dispensing unit with at least one nutrient medium container and a second medium is in fluid connection with its supply side by a gas dosing unit with at least one gas supply container. The cell culture space has a volume between 5 ml maximum and 0.1 ml minimum. The device also has an active substance supply container for supplying at least one active substance to the cell culture space, and creating an adjusted concentration-time curve of the active substance in the cell culture space.

[0035] In a preferred embodiment of the device of the invention, the first means are in a fluid connection with a waste container on the outflow side.

[0036] In a further preferred embodiment, the first means are in a fluid connection with the removal side by a recirculation line with the at least one nutrient medium container. In the line between the removal side of the first means and the waste container, a device may be installed that can isolate or detect certain metabolic products.

[0037] The first means located inside the interior of the cell culture container preferably consists of at least one membrane suitable for supplying liquid nutrient media.

[0038] The at least one membrane of the first means preferably comprises a semipermeable membrane suitable for adding a liquid nutrient medium, i.e., continuous material transport through the membrane wall can take place as a result of diffusive or convective transport mechanisms. Depending on the requirements, i.e.,

depending on whether diffusive or convective material transport of the nutrient medium is necessary, a nanofiltration, ultrafiltration, or microfiltration membrane is involved. Dialysis membranes such as CUPROPHAN<sup>®</sup> or hydrophilic microporous membranes such as microporous polyethersulfone membranes can be used, for example.

[0039] The second means located in the cell culture container preferably comprises at least one membrane suitable for gas exchange.

[0040] The at least one membrane of the second means preferably comprises of an oxygenation membrane, more preferably of at least an OXYPHAN<sup>®</sup> membrane.

[0041] According to the invention, the second means is in a fluid connection with a gas supply container. The gas supply container may comprise a gas chamber, for example, an incubator and the gas chamber and a second means are linked by a gas-permeable, sterile filter. In a preferred embodiment of the device of the invention, the second means has a fluid connection through at least one gas metering unit with at least one pressurized gas supply container. Different concentrations of various gas components can be adjusted in simple fashion.

[0042] In a preferred embodiment of the device of the invention, the membranes of the first and second means are made of hollow fibers.

[0043] More preferably, the hollow fibers are stacked in several layers in the inner space.

[0044] In another preferred embodiment, the maximum distance between the hollow fibers forming the respective means is between 50  $\mu\text{m}$  and 600  $\mu\text{m}$ .

[0045] In a preferred embodiment, the cell culture container has a bottom and a lid which bound the interior chamber, are located opposite one another, and are

made of a transparent material. The transparency of the bottom and the lid allows microscopic observation of the cells during the tests of the active substance.

[0046] In a more preferred embodiment, a heating system suitable for warming the cell culture container to 37°C is integrated into the bottom, for example, as a heating film which has sufficient transparency for viewing the cell culture, for example, by a microscope.

[0047] The cell culture space preferably has a volume of 0.3 to 3.0 ml. The advantage of this miniaturization consists in especially low use of cells, active substances, liquid, and gaseous media.

[0048] The means preferred in the device of the invention for adding the active substance comprises at least one active substance supply container, at least one active substance metering unit, and a system of pipes that connects the at least one active substance supply container through an active substance metering unit directly or through the first supply device with the cell culture space of the cell culture container.

[0049] In a preferred embodiment of the device according to the invention, the means for creating an active substance concentration-time curve in the cell culture space consists in the permeabilities of the membranes of the first means.

[0050] In a preferred embodiment, the device according to the invention comprises means for monitoring cell vitality.

[0051] A more preferred embodiment of the device of the invention comprises at least one sensor suitable for supplying information about the state of the cell culture as a means for monitoring cell vitality. Preferably, these are miniaturized sensors for determining proliferation, vitality, apoptosis, or generally cell death. More preferably, there is a sensor for detection of fluorescence.

**[0052]** The device of the invention may contain sensors suitable for monitoring the process, whereby preferably sensors are attached for temperature, pH, partial pressure of oxygen pO<sub>2</sub>, or carbon dioxide pCO<sub>2</sub>, glucose, or lactate located inside the cell culture container.

**[0053]** These sensors can be arranged individually or in combinations inside the cell culture container.

**[0054]** The goal of the present invention is also achieved by using a modular active substance test system comprising at least two of the devices of the invention.

**[0055]** Preferably, the modular active substance testing system comprises 6, 24 or 96 devices according to the invention which are arranged in a suitable manner to form a modular configuration.

**[0056]** Finally, the problem of the present invention is solved by a process for in-vitro testing of the effects of active substances on cells comprising the device according to the present invention and the modular active substance testing system according to the invention.

**[0055]** The device of the invention or the modular active substance testing system may be used to determine the influence pharmacokinetics on cell vitality.

**[0056]** The device of the invention or the modular effective substance testing system of the invention may be used to determine the influence of pharmacokinetics on the vitality of cells.

**[0057]** The present invention is explained in greater detail on the basis of the following drawings and the example. The following are shown in a simplified schematic representation:

**[0058]** Figure 1 is a flowchart of a device of the invention.

**[0059]** Figure 2a is a cross section through a modular active substance testing system with devices of the invention.

**[0060]** Figure 2b is a cross section through a modular active substance testing system with three stacked planes with devices of the invention. and

**[0061]** Figure 3 is a top view of a modular active substance testing system comprising six devices of the invention.

**[0062]** Figure 4a is an active substance concentration-time curve shown as Profiles 1 to 3.

**[0063]** Figure 4b shows the vital cells in four different cell culture containers as presented (inoculum) and as harvested cells (cell harvest).

**[0064]** Figure 1 shows a nutrient medium container 4, connected in a fluid manner by a pipe 14 and a nutrient medium dispensing unit 3 with the supply side of the first means contained in the interior chamber 2 of cell culture container 1. The removal side of the first means located in the interior chamber 2 of cell culture container 1 is connected in a fluid manner by line 14 with a waste container 10. In line 14 between the removal side of the first means and the waste container 10, there is a device 12 which can isolate or detect certain metabolic products. The recirculation line 11 permits a return of the fluid in interior chamber 2 of cell culture container 1 in nutrient medium container 4. An active substance supply container 7 is connected in a fluid manner by a line system 9, an active substance dispensing unit 8, and a line 9b with the supply side of the first means contained in the interior chamber 2 of cell culture container 1. In this configuration, an active substance reaches the interior chamber 2 of cell culture container 1 through the first means. The switching element 9c and line 9a permit a direct fluid connection between an active substance supply container 7 and interior chamber 2 of cell culture container 1. At least one gas

supply container 6 is connected in a fluid manner by a gas dispensing unit 5 with the supply side of the second means located in the interior chamber 2 of cell culture container 1, whose removal side is connected with a gas removal line 6a.

**[0065]** Figure 2a shows in cross section a modular active substance testing system mounted in a holder 13. A nutrient medium holder 4 is connected in a fluid manner with a nutrient medium line 14 by a nutrient medium dispensing unit 3 made as a tube pump, with the supply side of the first means 1a in the interior of cell culture container 1, with the connection between nutrient medium dispensing unit 3 and the supply side of first means 1a being in the form of lines 14 and 9b. The removal side of the first means 1a in the interior chamber of cell culture container 1 is connected in a fluid manner by a line 14 with waste container 10. However, the removal sides of a plurality of the devices according to the invention can be connected with a common waste container. Cell culture container 1 is placed on a baseplate 15 and fastened there by a latching mechanism. A heating film is integrated into baseplate 15 which is suitable for keeping the cell culture container and the medium at the required temperature, preferably 37°C, shortly before its entrance into the cell culture container. The heating film, however, can also be integrated into the bottom of the cell culture container 1 as long as this integration permits a sufficient transparency of the cell culture-containing bottom which is advantageous for a visual or microscopic observation of the cell culture. In front of and behind cell culture container 1, there are devices 16 suitable for sampling and can be made for example as a septum. The active substance supply container 7 is connected by line system 9, active substance dispensing unit 8, and line 9b with the supply side of first means 1a located in cell culture container 1. The lines carrying the fluids are preferably silicone tubing with an inside diameter of 1 mm.

**[0066]** Figure 2b shows a modular active substance testing system in cross section with three planes stacked in a holder 13 containing the devices of the invention. Figure 2b shows that the modular design of the devices of the invention provides a plurality of these devices so that many of the active substance tests can be performed simultaneously.

**[0067]** Figure 3 shows in top view a modular effective substance testing system in a holder 13 that holds six devices of the invention. Five of these devices are identical to the devices described in Figure 2a. The sixth device, shown at the very bottom in Figure 3, is a device 17 suitable for combination therapy, whose three active substance supply containers 18a, 18b, and 18c are connected in a fluid manner with the supply side of the first means by individual active substance pumps 19a, 19b, and 19c. Together with active substance supply container 7 and active substance dispensing unit 8, a combination therapy of four active substances can be performed in the cell culture container of the lowermost device of the invention shown in Figure 3.

**[0068]** The devices of the modular effective substance testing system isolate nutrient media, cell cultures, active substances, and waste solutions from the outside world. All of the parts of the device that come in contact with the active substance are preferably made as disposable articles. Therefore, each device in the modular system can be removed individually by the above-mentioned latching mechanism from this system. It is not necessary for the operating personnel to then come in contact with the partially highly toxic active substances. All of the parts that come in contact with the nutrient medium, active substance, and cell culture must be sterilizable. Sterile operation of the device for 10 days has been demonstrated.

**[0069]** Thus, it becomes clear that the modular arrangement of the devices allows a very high number of active substance tests with different pharmacokinetics

and active substance combinations and the possibility of the modular system helps to choose the number and layout of the individual devices in many different ways. For example, reference devices without the addition of active substances can be operated in parallel with devices with active substances. The modular design of the system, consisting of individual devices, has the additional advantage over conventional cell culture vessels (96-wave plates, 24-wave plates, and 6-wave plates) of individual manipulability of the individual devices. However, the same manipulations (same sample, same treatment) can be performed in different channels (multiple measurements).

[0070] A modular effective substance testing system consisting of six devices weighs less than 10 kg and can be easily worn by an individual. Owing to the small dimensions, for example, a modular effective substance testing system consisting of 24 devices can be operated even in a conventional CO<sub>2</sub> incubator.

[0071] A personal computer is usually employed for system control, sample identification, sample collection, and data evaluation, on whose screen the current measured values can be followed. It is also possible to compare data between individual channels. The evaluation software can handle trend analysis and analyze the difference between reference and active substance channels. The results can be evaluated for individual patients and then stored.

[0072] The following example shows how the modular effective substance testing system can be used to measure the influence of pharmacokinetics on the vitality of cells.

[0073] Example:

[0074] The leukemic cell line CCRF CEM was supplied in a density of  $1 \cdot 10^7$  per ml of cell culture medium (RPMI 1640 and 10 vol.% fetal calf serum based on

RPMI 1640) and in a volume of 300  $\mu$ l in four cell culture containers of a modular active substance testing system according to the invention consisting of four devices according to the invention.

**[0075]** The modular active substance testing system was enclosed in an incubator in which a temperature of 37°C and a gaseous medium consisting of 5% CO<sub>2</sub>, 74% N<sub>2</sub>, and 21% O<sub>2</sub> was provided. The addition of the gaseous medium just mentioned takes place diffusively using sterile filters via the second membrane system made of OXYPHAN® into the interior of cell culture containers 1 to 4. RPMI 1640 and 10 vol.% of total calf serum based on RPMI 1640 was used as the nutrient medium. For a time of 24 h, the nutrient medium was recirculated at a fluid rate of 7 ml/min, so that the leukemic cell lines were supplied with nutrient medium by the membranes of the first membrane system in the form of hollow fibers made of CUPROPHAN®. After 24 h of recirculation, the nutrient medium supply was interrupted and cytostatic idarubicin was added with three different active substance concentration-time curves with a fluid flow rate of the nutrient medium of 0.2 ml per minute over the CUPROPHAN® membranes in these cell culture containers of the devices according to the invention as described in the following:

**[0076]** The active substance concentration-time curves are shown in Figure 4a) as Profiles 1 to 3.

**[0077]** Profile 1: A solution of 0.20  $\mu$ g of idarubicin per ml of the above-mentioned cell culture medium was fed through the CUPROPHAN® hollow fibers of cell culture container 1 in a period of 75 minutes.

**[0078]** Profile 2: A solution of 0.50  $\mu$ g idarubicin per ml of the above-mentioned cell culture medium was conducted for 20 minutes through the hollow

CUPROPHAN® fibers of cell culture container 2. Then a solution of 0.25 µg idarubicin per ml of the above-mentioned cell culture medium was conducted through the hollow CUPROPHAN® fibers of cell culture container 2 for 20 minutes.

**[0079]** Profile 3: A solution of 1.00 µg idarubicin per ml of the above-mentioned cell culture medium was conducted through the hollow CUPROPHAN® fibers of cell culture container 3 for 15 minutes.

**[0080]** No idarubicin was added to the interior of cell culture container 4. This cell culture container served as a control. The administration of the active substance took place in such a way that for all of the active substance concentration-time curves described above, the same area under the curve (AUC) resulted. After adding the cytostatic agent the cell culture container was flushed with fresh nutrient medium (RPMI 1640 and 10 vol.% fetal calf serum based on RPMI 1640) for 1 hr, while the nutrient medium was conducted at a flowrate of 0.2 ml/min through the membranes of the first membrane system and the fluid flow emerging from the membranes was conducted into the respective waste containers. Then nutrient medium recirculation was resumed at a flowrate of 7 ml/min. After 72 hr, the previously used nutrient medium was replaced by the same but fresh nutrient medium. After 96 hr, the cells from the four cell culture containers were harvested and the number of vital cells was determined by Trypan Blue dye and as the vitality the percentile component of the number of vital cells harvested to the total number of harvested cells was determined according to the following relationship:

**[0081]** 
$$\text{Vitality} = (\text{number of harvested vital cells} / \text{total number of harvested cells}) \cdot 100\%.$$

**[0082]** In Figure 4b, the word "inoculum" is followed by the number of vital cells supplied in cell culture containers 1 to 4. Since the cells were supplied in a

volume of 300  $\mu\text{l}$  and in a cell density of  $1 \cdot 10^7$  per ml of cell culture medium, the number of vital cells supplied in the cell culture containers was  $1$  to  $4 \cdot 30 \cdot 10^5$ . The words "cell harvest" are followed in Figure 4b by the number of vital cells obtained after the cell harvest. It is clear that the active substance concentration-time curve according to Profile 1 reduced the number of vital cells the most.

**[0083]** The vitalities of the cells from cell culture containers 1 to 4 were obtained with the following results:

Cell culture container	Vitality
1	29%
2	36%
3	47%
4	90%

Attachment to the filing of February 9, 2001

Claims:

1. Use of a method comprising at least the following steps:

a) providing a cell culture container with an interior space and an inside wall and with a first and second membrane system located in the interior space, whereby a cell culture space is formed between the membrane systems and the inside wall of the interior chamber;

b) providing cells as a cell culture and a cell culture medium in the cell culture chamber;

c) adding a fluid nutrient medium to the cell culture chamber and removing metabolic products from the cell culture chamber by means of the first membrane system;

d) adding at least one gaseous medium to the cell culture chamber by means of a second membrane system;

e) metering at least one active substance into the cell culture chamber, with the metering taking place according to an adjusted active substance concentration-time curve; and

f) monitoring cell vitality.  
for in-vitro testing of active substances in cells.

2. Use according to Claim 1, characterized in that cytostatics, antibiotics, cytokines, growth factors, or antiviral agents are used as active substances.

3. Use according to one or more of Claims 1 or 2, characterized in that primary cells are added as the cell culture.
4. Use according to one or more of Claims 1 or 2, characterized in that tumor cell lines are used as the cell culture.
5. Use according to one or more of Claims 1 to 4, characterized in that the cell culture chamber has a volume of at least 0.1 ml minimum and 5 ml maximum.
6. Use according to Claim 5, characterized in that the cell culture chamber has a minimum volume of 0.3 ml and a maximum volume of 3.0 ml.
7. Use according to one or more of Claims 1 to 6, characterized in that at least one semipermeable membrane or at least one hydrophilic microporous membrane is used as the first membrane system and at least one gas transfer membrane is used as the second membrane system.
8. Use according to one or more of Claims 1 to 7, characterized in that the first and the second membrane systems consist of hollow fibers stacked in multiple layers.
9. Use according to one or more of Claims 1 to 8, characterized in that a cell culture container is used which has a removable lid and allows the cell culture to be prepared by adjusting the desired cell density in the cell culture medium, opening the lid of the cell culture container, pipetting the desired volume of cell suspension into the cell culture container, and closing the cell culture container using the lid.
10. Use according to one or more of Claims 1 to 9, characterized in the fact that RPMI 1640 is used as the cell culture medium.
11. Use according to one or more of Claims 1 to 10, characterized in that at least  $1 \cdot 10^5$  cells per ml of cell culture space are used.

12. Use according to one or more of Claims 1 to 11, characterized in that each cell is at an average distance of 0  $\mu$ m to 600  $\mu$ m from the closest membrane in the first and second membrane systems.

13. Use according to one or more of Claims 1 to 12, characterized in that a fluid nutrient medium RPMI 1640 is used.

14. Use according to one or more of Claims 1 to 13, characterized in that the gaseous medium has a  $pO_2$  of 0 to 160 mmHg and a  $pCO_2$  of 0 to 115 mmHg.

15. Use according to one or more of Claims 1 to 14, characterized in that the cell culture medium contains a bicarbonate buffer and the  $pCO_2$  in the gaseous medium added is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

16. Use according to one or more of Claims 1 to 15, characterized in that gaseous metabolic products are removed from the cell culture space by means of the second membrane system.

17. Use according to one or more of Claims 1 to 16, characterized in that individual active substances and/or combinations of several active substances are added on a time-staggered basis.

18. Use according to one or several of Claims 1 to 17, characterized in that the active substance dosage is added to the cell culture chamber directly or by means of the first membrane system.

19. Use according to one or more of Claims 1 to 18, characterized in that specification of the active substance concentration-time curve takes place with the permeabilities of the first membrane system, by the duration of the active substance administration, and by the active substance concentration.

20. Use according to one or more of Claims 1 to 19, characterized in that the cell culture container is kept at 37°C.

21. Use according to one or more of Claims 1 to 18, characterized in that the cell vitality is monitored by means of a cell vitality dye.

22. Use according to Claim 21, characterized in that Alamar Blue<sup>®</sup> serves as a cell vitality dye.

23. Use according to one or more of Claims 1 to 22, characterized in the fact the cell vitality is monitored using at least one sensor.

24. Use according to Claim 23, characterized in that a fluorescence sensor is used.

25. Device for in-vitro testing of active substances in cells, comprising a cell culture container (1) suitable for collecting a cell culture in a cell culture medium with an internal chamber (2), with first means for supplying at least one nutrient medium and second means for adding at least one gaseous medium located in the interior space, with the means each having a supply side and a removal side, and with a cell culture space being formed between said means and the inside wall of the interior chamber, and with the first means in a fluid connection with the supply side connected by nutrient medium dispensing unit (3) with at least one nutrient medium container (4), and the second means connected in a fluid connection with the supply side connected by a gas metering unit (5) with at least one gas supply container (6), characterized in that the cell culture chamber has a volume of at most 5 ml and at least 0.1 ml, and that the device also contains means (7), (8), (9a), (9b), and (9c) for supplying at least one active substance to the cell culture chamber and means for creating an active substance concentration-time curve in the cell culture chamber.

26. Device according to Claim 25, characterized in that the first means is in a fluid connection on the removal side with a waste container (10).

27. Device according to Claim 25, characterized in that the first means is in a fluid connection on the removal side by a recirculation line (11) with the at least one nutrient medium container (4).

28. Device according to one or more of Claims 25 to 27, characterized in that the first means consists of at least one fluid medium suitable for administration.

29. Device according to one or more of Claims 25 to 28, characterized in that the second means consists of at least one membrane suitable for gas exchange.

30. Device according to one or more of Claims 25 to 29, characterized in that cell culture container (1) has a bottom and a lid which bound the interior chamber, are opposite one another, and consist of transparent material.

31. Device according to Claim 30, characterized in that a heating system is integrated into the bottom of cell culture container (1).

32. Device according to one or more of Claims 25 to 31, characterized in that the at least one membrane of the first means is a semipermeable membrane or a hydrophilic microporous membrane.

33. Device according to one or more of Claims 25 to 32, characterized in that the at least one membrane of the second means is an oxygenation membrane.

34. Device according to one or more of Claims 25 to 33, characterized in that the membranes of the first and second means are hollow fibers.

35. Device according to one or more of Claims 25 to 34, characterized in that the hollow fibers are stacked in several layers in the interior chamber.

36. Device according to Claim 35, characterized in that the maximum distance between the hollow fibers forming each means is between 50  $\mu$ m and 600  $\mu$ m.

37. Device according to one or more of Claims 25 to 36, characterized in that the cell culture chamber has a volume of 0.3 ml to 3.0 ml.

38. Device according to one or more of Claims 25 to 37, characterized in that the means for adding the active substance consist of at least one active substance supply container (7), at least one active substance metering device (8), and a system of lines (9) which connects the at least one active substance supply container (7) through an active substance metering unit (8) directly (9a) or through first means (9b) with the cell culture chamber of cell culture container (1).

39. Device according to one or more of Claims 25 to 38, characterized in that the device has a means for monitoring cell vitality.

40. Device according to Claim 39, characterized in that the means for monitoring cell vitality consists of at least one sensor.

41. Device according to Claim 40, characterized in that the sensor is a fluorescence sensor.

42. Modular active substance testing system comprising at least two devices according to Claims 25 to 41.

43. Modular active substance testing system according to Claim 42, consisting of 6, 24, or 96 devices according to Claims 25 to 41.

44. Use of the device according to one or more of Claims 25 to 41 or of the modular active substance testing system according to one of Claims 42 or 43 for in-vitro testing of the effects of active substances on cells.

45. Use of the device or of the modular system according to Claim 44, characterized in that the influence of pharmacokinetics on cell vitality is determined.

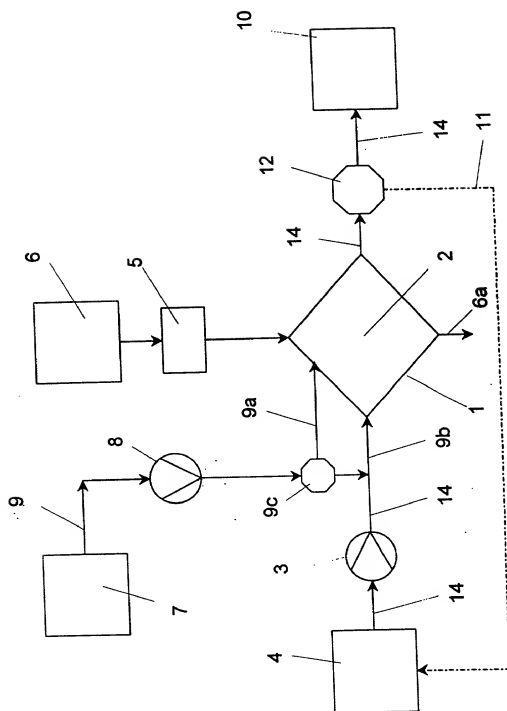
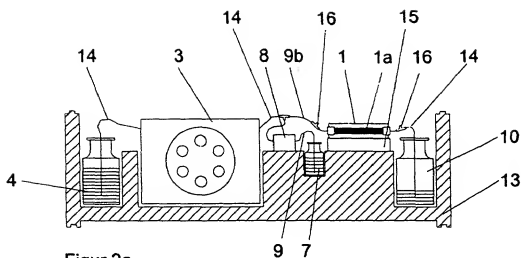
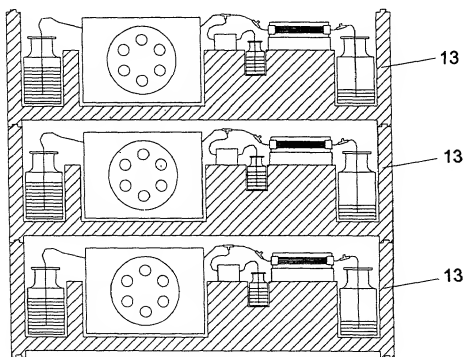


Figure 1  
Figure

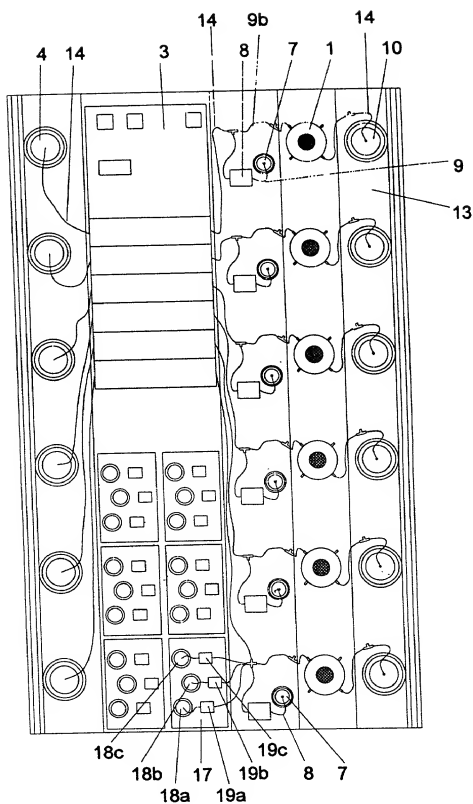
2/4



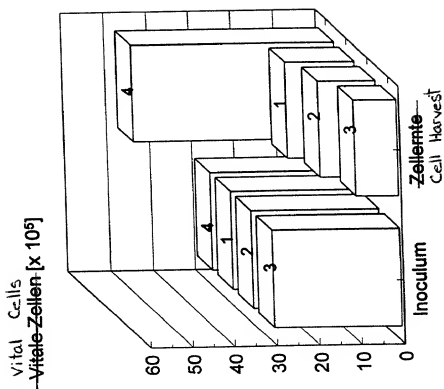
Figur 2a  
Figure



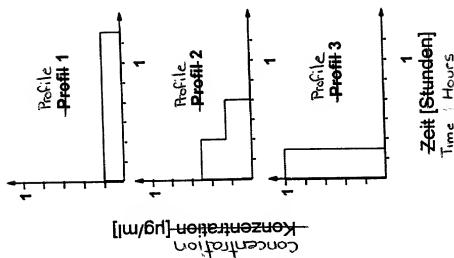
Figur 2b  
Figure



**Figur 3**  
Figure



Figur 4 b  
Figure



Figur 4 a  
Figure

**DECLARATION AND POWER OF ATTORNEY  
UNDER 35 USC §371(c)(4) FOR  
PCT APPLICATION FOR UNITED STATES PATENT**

As a below named inventor, I hereby declare that:  
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: Method for the In Vitro Testing of Active Ingredients Corresponding  
Device and Their Use

described and claimed in international application number PCT/EP00/02011 filed March 8, 2000.

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

German Patent Application No. 19910540.5 filed March 9, 1999

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William F. Berridge, Reg. No. 30,024;  
Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411;  
Edward P. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771;  
Mario A. Costantino, Reg. No. 33,565; Stephen J. Roe, Reg. No. 34,463;  
Joel S. Armstrong, Reg. No. 36,430; Christopher W. Brown, Reg. No. 38,025; and  
Richard E. Rice, Reg. No. 31,560.

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	Typewritten Full Name of Sole or First Inventor	Herma Given Name	GLOCKNER Middle Initial	GLOCKNER Family Name
2	Inventor's Signature	<u>Herma Glockner</u>		
3	Date of Signature	09 Month	25 Day	2001 Year
	Residence:	Kleinwallstadt City		GERMANY Country
	Citizenship:	German		
	Post Office Address: (Insert complete mailing address, including country)	Dammweg 5 63839 Kleinwallstadt, GERMANY		

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☒  
(Discard this page in a sole inventor application)

1 **Typewritten Full Name  
of Joint Inventor**

2 **Inventor's Signature:**

3 **Date of Signature:**

Residence:

Citizenship:

Post Office Address:  
(Insert complete mailing  
address, including country)

Horst-Dieter

Given Name

Middle Initial

LEMKE

Family Name

09

Month

25

Day

2001

Year

Obemburg

City

State or Province

GERMANY

Country

Dr.-Kittlweg 6

63785 Obemburg, GERMANY

1 **Typewritten Full Name  
of Joint Inventor**

2 **Inventor's Signature:**

3 **Date of Signature:**

Residence:

Citizenship:

Post Office Address:  
(Insert complete mailing  
address, including country)

Christoph

Given Name

Middle Initial

MEYER

Family Name

09

Month

27

Day

2001

Year

Saarbrücken

City

State or Province

GERMANY

Country

Waldwiese 5

66123 Saarbrücken, GERMANY

1 **Typewritten Full Name  
of Joint Inventor**

2 **Inventor's Signature:**

3 **Date of Signature:**

Residence:

Citizenship:

Post Office Address:  
(Insert complete mailing  
address, including country)

Given Name

Middle Initial

Family Name

Month

Day

Year

City

State or Province

Country

1 **Typewritten Full Name  
of Joint Inventor**

2 **Inventor's Signature:**

3 **Date of Signature:**

Residence:

Citizenship:

Post Office Address:  
(Insert complete mailing  
address, including country)

Given Name

Middle Initial

Family Name

Month

Day

Year

City

State or Province

Country

**Note to Inventor:** Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

This form may be executed only when attached to the first page of the Declaration and Power of Attorney of the application to which it pertains.